A STUDY OF OVARIAN ACTIVITY IN THE PREGNANT WOMAN.

by

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PREFACE.

The investigations on which this thesis is based were carried out in the Wards and Research Department of the Royal Maternity and Women's Hospital, Glasgow.

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INTRODUCTION.

It is commonly thought that, apart from the corpus luteum, the ovaries are inactive during pregnancy. Even in this respect, it is tacitly assumed that the corpus luteum ceases to function around the twelfth week of pregnancy, and thereafter undergoes degeneration and disappears. In view of this, it is not surprising to find that although a voluminous literature has accumulated, relating to the association of ovarian activity with pregnancy, almost all of it deals with the corpus luteum (Loeb, 1910; Williams and Williams, 1921; Hammond, 1927).

However, in spite of the fact that the ovary in pregnancy is said to be inactive, there are several observations which do not quite fit in with this conception. For example, in the conditions of hydatidiform mole and chorionepithelioma, bilateral multiple lutein cysts of the ovary are often found. (Cottalorda, 1921; Selye, 1948; Mahfouz Pasha and Ismail, 1941).

More recently, however, there have been a number of investigations in animals, which indicate that the ovary is not inactive in later pregnancy. Studies in lower animals have shown that the corpus luteum persists, (Nelson, 1929; Swezy and Evans, 1930; Swezy, 1933; Gillman and Stein, 1941), and according to van der Horst and Gillman (1940) it passes through several phases of activity. In addition, contrary to the popular idea that ovarian activity, if any, is confined to the corpua luteum, it has been found that in animals, at least there is a considerable degree of follicular maturation, if not ovulation, during pregnancy (Swezy, 1933; van der Horst and Gillman, 1941; van der Horst, 1944a). It has been claimed by Gillman (1941), that similar follicular maturation occurs in the human.

It is vitally important to determine whether the changes found in animals are similar to those occurring in humans. So many theories have been built on the assumption that the ovaries are inactive, and that their hormonal function

at least, is taken over by the placenta. Nevertheless, these ovarian changes, if present in the human, must represent the results of endocrine activity, and doubtless would result in some degree of hormonal secretion by the ovary. Two primary problems therefore present themselves. Chorionic gonadotrophin undoubtedly exists, and has been estimated in the blood, urine, placenta, and in cultures of placental tissue (Aschheim, 1926, 1927; Zondek and Aschheim, 1927; Friedheim, 1929; Smith and Smith, 1935; Sengupta, 1935; Evans, 1936; Gey, Seegar Jones and Hellman, 1938; Smith and Smith, 1939, 1940, 1941; Rydberg and Pedersen-Bjergaard, 1943; Wislocki and Bennett, 1943; Seegar Jones, Gey andGey, 1943; Wislocki, Dempsey and Fawcett, 1948; Stroink and Muhlbock, 1948; Stewart, Sano and Montgomery, 1948; Loraine and Matthew, 1950).

It has been stated, however, that chorionic gonadotrophin has little effect on the human ovary, and therefore one would scarcely expect the ovary to show much activity during pregnancy. On the other hand, a number of investigators have

suggested that some, at least, of the gonadotrophic activity exhibited during pregnancy, is the result of pituitary secretion (Zondek, 1932; Schockaert and Siebke, 1933; Kunkel and Loeb, 1935; Fremery and Scheygrond, 1937; Witschi, 1937; Govan and Mukherjee, 1950; Lajos, Szontagh and Pali, 1953; Calapa, 1954), which would naturally have an influence on ovarian structure and function.

The steroid hormones, usually produced by the ovary, play a prominent part in the physiology of the pregnant organism. High levels of oestrogens have been found in the blood, placenta, urine, and faeces, (Cohen and Marrian, 1934; Marrian and Newton, 1935; Cohen, Marrian, and Watson, 1935; Robson, 1936). In addition to these workers, the estimation of oestrin was carried out by most of the workers mentioned in connection with the estimation of chorionic gonadotrophins.

Comparatively little work has been done on the excretion of progesterone, as only biological methods are so far available. However,

many workers have estimated the excretion of the metabolic product of progesterone - pregnanediol. Unfortunately, pregnanediol is the result of the excretion of several hormones, and so it is possible that the conclusions drawn from these investigations may not be completely valid, (Browne, Henry and Venning, 1937; Jones and Weil, 1938; Cope, 1940; Bachman, Leekley and Hirschmann, 1940; Tien, 1941; Talbot et al., 1941; Astwood and Seegar Jones, 1941; Guterman, 1944, 1945, 1946; Venning, 1946; Jayle and Libert, 1946; Dahle, 1946; McCormack, 1946; Somerville, Gough and Marrian, 1947; Bender, 1947; Bishop, 1949; Boll.Soc.Ital.Biol. 1952; Bradshaw, 1953).

In early pregnancy, progesterone is secreted by the ovary, but later in pregnancy, this hormone appears to be secreted by the placenta (Robson, 1936; Seegar and Delfs, 1940; Wislocki, Dempsey and Fawcett, 1948). Oestrogens, also, are secreted by the ovary in early pregnancy, and, like progesterone, have been found in the placenta, in later pregnancy. (Stroink and

Muhlbock, 1948; Wislocki, Dempsey and Fawcett, 1948; Stewart, Sano and Montgomery, 1948).

If, however, the ovary is active throughout pregnancy, it is possible that many of the steroids previously thought to be of placental origin, may be derived from the ovaries.

A further important point that arises, is that in certain conditions, e.g. pre-eclamptic toxaemia, hyperemesis gravidarum; acute yellow atrophy of the liver occurring in pregnancy, etc., there are thought to occur, major changes in the hormonal pattern. (Anselmino, Hoffman and Kennedy, 1932; Heim, 1935; Cushing, 1934; Seigler, 1938, 1939; Smith and Smith, 1934, 1935, 1938, 1939; Stroink and Muhlbock, 1948; Loraine and Matthew, 1950; Govan, 1951; Boll.Soc.Ital. Biol.Sper., 1952; Lajos, Szontagh and Pali, 1953; Agadzhanoff, 1953). It has been shown that in many of these conditions, but especially in toxaemias of pregnancy, there is a deficiency of both oestrogens and progesterone.

In pregnancy associated with diabetes mellitus, it has been found that the chorionic

gonadotrophin levels are abnormally high, along with a low urinary pregnanediol, and a low serum oestrogen. (White, 1937; White, Titus, Joslin and Hunt, 1939; White, 1943; White and Hunt, 1943; White, 1946; 1947; Loraine, 1949). In connection with the hormonal changes in these various conditions, it is of fundamental importance to discover the origin of these hormones. From the above, several points require clarification:-

(1) It is essential to determine whether the corpus luteum degenerates and disappears, as is commonly held, at the third to fourth month of pregnancy, or whether, as is stated by observers, studying animal tissues, it persists throughout pregnancy, and shows a varying degree of activity from time to time.

(2) In addition, there is a need for an investigation of the activity of the ovary in late pregnancy. The common opinion is that the ovary is inactive, and relatively atrophic towards the end of pregnancy, but there is some evidence which controverts this traditional outlook.

(3) It would be interesting to determine the nature of such activity, if it exists, assuming for the moment that there is ovarian activity. It would suggest that the gonadotrophic hormones of late pregnancy differ qualitatively from those of early pregnancy.

In view of the confusion which has arisen in regard to hormonal activity in pregnancy, it was decided to study, as far as possible, the changes, if any, occurring in the human ovary during the various stages of pregnancy. In addition, a study of the exact nature of the influence of urinary gonadotrophins on ovarian structure has been made. This has been carried out by extracting these hormones from the urine of patients at various stages of pregnancy and studying their effects on the ovaries of miniature animals.

In addition, an attempt was made to assess the output of ovarian steroids by a simple technique.

<u>The following is an attempt to answer some of these questions.</u>

It was determined to study ovaries taken

from pregnant women, either from those who had died undelivered, or had been subjected to some kind of operative interference, usually Caesarean Section or ectopic pregnancy. In addition there were a small series of women dying in early pregnancy, and one who had died three months after delivery.

The total number of cases studied was , and this number has been made up as follows:-

(1) Specimens from post-mortems from women dying in early pregnancy - 7.

(2) Specimens from women in early pregnancy, obtained at laparotomy for ectopic pregnancy - 18.

(3) Specimens from post-mortems from women dying in later pregnancy - 42.

(4) Specimens from post-mortems from women dying three months after delivery - 1.

(5) Specimens obtained at Caesarean Section operations performed on women at full-term pregnancy - 20.

The details of these groups are as follows:-

Early pregnancy, up to 18 weeks - 25.

Pregnancy at 28 weeks - 1.

Pregnancy at 30 weeks - 4.

Pregnancy at 33 weeks - 5.

Pregnancy at 36 weeks - 5.

Pregnancy at 40 weeks - 27.

After removal, the ovaries were fixed in formol-corrosive, and embedded in paraffin. Sections of the specimens were cut at a thickness of 5 μ, and they were stained with Haemalum and Eosin.

In a later section of the thesis, dealing with gonadotrophic hormones, the details of experiments performed, are given.

All the sections examined, were studied in the same way, with regard to the following points of importance:-

(1) The presence and condition of a corpus luteum, or else the presence of a corpus albicans, or corpora albicantia, if not corpus luteum was present.

(2) A description of the developing follicles, in various stages of maturation.

(3) A description of the follicles which had gone

on to atresia. These have been described as either patent or collapsed, and details are given of the histological structure of the tissue lining the follicle.

(4) Histological detail of the theca is also described. This is classified according to (a) degree of proliferation; (b) vascularity.

(5) The presence of areas of thecal change is described. These are regarded as areas composed of tissue similar to that lining the atretic follicles, but which could not be identified as composing part of a follicle.

(6) Areas of decidual change are described, when they occur. A description, according to the above plan, will now be given of the different classes of cases, named previously.

(A) Early Pregnancy.

Specimens were available from seven patients who had died in the early months of pregnancy. The actual duration of these pregnancies was as follows:-

> Twelve weeks; eight weeks; seventeen weeks; eighteen weeks; twelve weeks; sixteen weeks; sixteen weeks.

Unfortunately, in three cases, only specimens from one ovary was available for study.

(1) The Corpus Luteum.

In this group there was a corpus luteum present in four of the ovaries each having been taken from the cases where both ovaries had been removed. In each specimen there was evidence of degeneration of this structure, although the degree of degeneration was not uniform. In two of the specimens, there was only slight degenerative change which was shown by the disappearance of some nuclei, along with breaking up of other nuelci. There was also definite diminution in the staining intensity of the nuclei, but no free chromatin material was present.

In the other two specimens, there was more marked degenerative change in the corpora lutea. This was evidenced by the disappearance of many nuclei, with breaking up of many others. There was a marked diminution in the staining intensity of the nuclei, along with the presence of free chromatin material. In one of the specimens showing the presence of a corpus luteum,

there was, in addition, one corpus albican. In the other three specimens, there were several corpora albicantia of varying sizes, although all were small. In the three specimens, which did not show the presence of a corpus luteum, one did not show any corpus albicans; one showed the presence of one corpus albicans, and one showed the presence of several corpora albicantia.

Apart from the corpus luteum, there was obvious stimulation of follicles in other parts of the ovary. Perhaps the most striking feature was the development of atretic follicles, lined by a well defined layer of theca interna. At this point, it is necessary to provide certain definitions which will aid in the description of these and later specimens. Since it is sometimes difficult without close study to state the origin of certain cells in the Graafian follicles, it is essential first of all, to describe the granulosa and thecal layers.

The Granulosa cells are irregular in shape, with eosinic cytoplasm. They are larger than the thecal cells. The nuclei are round or oval, and

are larger and less deeply staining, than the nuclei of the thecal cells. They show an obvious chromatin network, although this is finer than in the case of the thecal nuclei. There are chromatin knots present, although these are fewer in number than in the case of the thecal nuclei.

The Thecal cells are also irregular in shape, with eosinic cytoplasm. They are smaller than the granulosa cells. The nuclei are round or oval, and are smaller and more deeply staining than the nuclei of the granulosa cells. They also show an obvious chromatin network, although this is coarser than in the case of the granulosa nuclei. Most of them show about two to four chromatin knots.

Even with this picture, it is still sometimes rather difficult to differentiate these layers, particularly if the granulosa is degenerating. However, another aid to differentiation is the existence of a single layer of spindle cells, which separates the granulosa from the theca interna.

(2) Developing Follicles.

These were seen in different stages of maturation, which have been described as follows:-

(a) Primordial follicles - these are composed of an ovum surrounded by a single layer of flattened cells.

(b) Relatively more advanced - these are composed of an ovum surrounded by several layers of granulosa cells.

(c) Fully developed follicles - these consist of a large follicle lined by theca interna tissue, internal to which is a layer of granulosa tissue. This granulosa tissue lines a large antrum and also forms a cumulus, in which the ovum is usually embedded. In addition to these fully developed follicles, there are occasionally follicles, which, while showing the same characteristics, are only about half the size of the first developed variety.

The first specimen showed the presence of twelve primordial follicles, all composed of an ovum, surrounded with a layer of flattened cells.

There was also one fully developed follicle, with an ovum present in the cumulus.

The second specimen showed the presence of eight primordial follicles, all consisting of an ovum surrounded by a layer of flattened cells. No further advanced follicles were present.

The third specimen showed only four early primordial follicles.

The fourth specimen showed only one early primordial follicle present in the entire section.

The fifth specimen showed ten early primordial follicles, seven of them being surrounded with a single layer of flattened cells, while the other three were surrounded with about three layers of granulosa cells.

The sixth specimen did not show the presence of any developing follicles at all.

The seventh specimen showed the presence of 31 primordial follicles. They were all surrounded with a single layer of flattened cells, and almost all of them contained an ovum.

There was also one follicle which was much more mature. It was about half the size of a

Figure 1. This shows a developing follicle, with well-marked thecal reaction, from a case of late pregnancy.

fully developed follicle,* with a granulosa layer, several cells thick. There was a well-marked antrum, and cumulus formation was commencing around the ovum.

(3) Atretic Follicles.

In addition to the presence of corpora lutea, in these early pregnancy specimens, the other main feature of interest, was the presence of many atretic follicles. These varied greatly in size, some being small, while others were very large. Some were patent, while others were collapsed. Whether patent or collapsed, all were lined with theca interna tissue, and in addition, in some was granulosa tissue also. This was almost always clearly identified by the presence of Call-Exner bodies.

Atretic follicles, in the seven sections taken from early pregnancy cases, showed these features:-

The first specimen showed eight atretic follicles, all of which were patent, varying in size, from small to large. These were lined with theca interna.x This was not degenerate, in

* See Figure 1.
x See Figure 2.

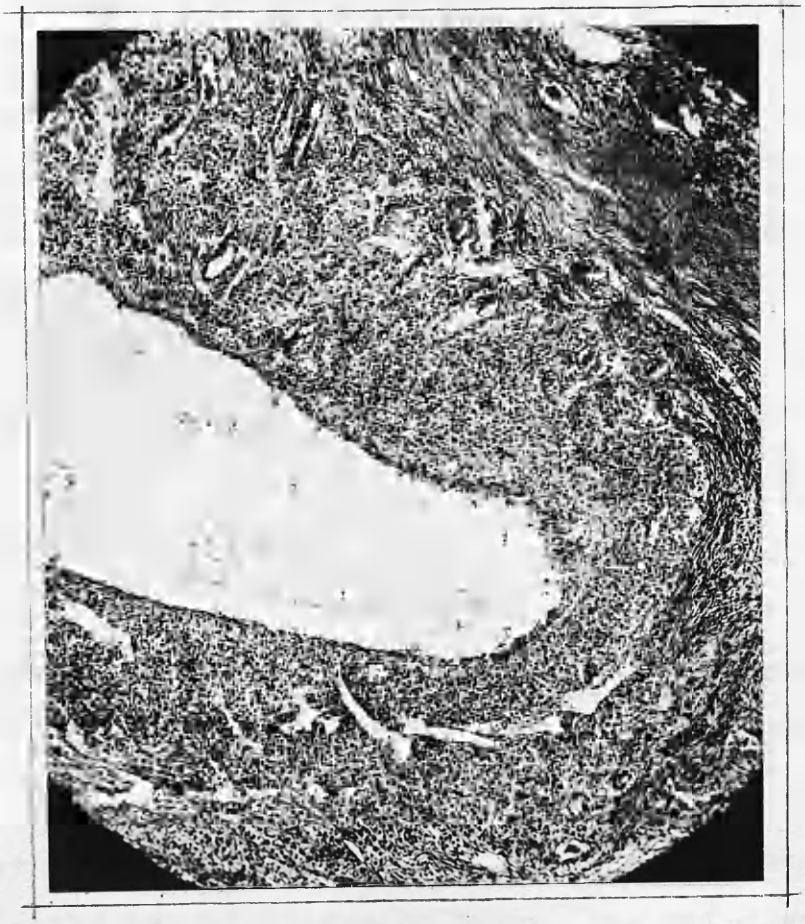

Figure 2. Low power view of atretic follicle, with hypertrophic thecal layer.

most of the specimens, but in one follicle, degenerative changes were well marked, the theca next to the cavity, being replaced with a layer of hyaline material.

The second specimen showed the presence of two atretic follicles, one being patent and the other collapsed. The patent follicle was moderately large.

In neither follicle was there any trace of granulosa, and both were lined with theca interna. This showed only moderate degenerative changes.

The third specimen showed the presence of three atretic follicles. These were all patent, and while two of them were large, the third was small. The three follicles were all lined with theca interna. This showed only slight degenerative changes in one of the large follicles, and the small one, but in the other large follicle, most of the theca had disappeared, and was being replaced by hyaline material.

In the large follicle, whose theca did not show marked degenerative changes, there was also a well-defined granulosa layer, lying detached in

the lumen of the follicle.

The fourth specimen showed one atretic follicle, which was patent. This was lined with theca interna, no trace of granulosa tissue remaining. The theca showed well-marked degenerative changes, with disappearance of some nuclei, and breaking up of others. Towards the lumen of the follicle, the theca was replaced by a layer of hyaline material.

The fifth specimen showed the presence of two patent atretic follicles. Both were lined with theca interna, and lying free, in the lumen of both, was granulosa tissue. Both the theca and the granulosa showed well-marked degenerative changes. These consisted of disappearance of nuclei, with loss of staining intensity of the other nuclei. There was also free chromatin material present.

The sixth specimen did not show the presence of any atretic follicles.

The seventh specimen showed the presence of six, patent, atretic follicles. All the follicles were lined with theca interna, while in

two of them, there was a well-marked granulosa layer, in situ, internal to the theca interna.

Neither the theca nor the granulosa layers showed degenerative changes, but in two of the follicles lined by theca alone, there was a thin layer of fibroid tissue, lining the cavity. There was also one collapsed, atretic, follicle. This also was lined with theca interna, which was not degenerate.

(4) Histological detail of the Theca.

The activity of the theca interna was judged on two criteria:-

(a) The degree of proliferation - this was estimated by a visual estimation of the thickness of the wall of the follicle. While a moderate degree of thickness was usually present, some follicles showed this feature to such a degree, that the area between two adjacent follicles would be completely occupied with a solid mass of theca.

(b) Increased vascular change - this was estimated by the presence of a considerable number of blood vessels, this feature not being

Figure 3. Higher power view of follicle as in Figure 2, showing the highly vascular nature of the hypertrophic thecal layer.

common to all the follicles.

The first specimen did not show proliferation of any marked degree, or any increased vascular change.

The second specimen showed a marked degree of proliferation, and increased vascularity was also present.*

The third specimen showed a moderate degree of proliferation, but there was no increased vascular change.

The fourth specimen again showed a marked degree of proliferation, but there was no increase in vascularity.

The fifth specimen showed a moderate degree of proliferation, but again without increased vascularity.

The sixth specimen as mentioned before, did not contain any atretic follicles.

The seventh specimen showed a moderate degree of proliferation, and increased vascular change was well marked.

(5) Areas of Thecal Change.

In addition to the thecal cells lining the

* See Figure 3.

follicles, there were isolated solid masses of thecal cells. These had the same histological features as those of the theca interna lining the atretic follicles, but were scattered throughout the ovarian stroma without apparent relationship to follicular activity.

In the sections taken from early pregnancy cases, no such areas of thecal change were seen.

(B) The eighteen cases obtained at laparotomy, for ectopic gestation in early pregnancy.

In these eighteen cases, the duration of pregnancy varied, but in every case, was less than seventeen weeks.

Unfortunately , as will be readily understood, in each case, only specimens from one ovary were available for study.

(1) The Corpus Luteum.

In this group there was a corpus luteum present in nine of the eighteen cases studied. In eight of these cases, it was large, while in the other case, it was small. In all the specimens except one, there was evidence of degeneration of this structure, although the degree of degeneration was not uniform. As stated, one of the specimens did not show the presence of any degenerative changes, while in three of the specimens, there were only slight degenerative changes. These changes consisted of disappearance of some nuclei, along with breaking-up of other nuclei.

In five of the specimens, there was more marked degenerative change in the corpora lutea.

This was shown by the disappearance of many nuclei, with breaking up of many others. There was a marked diminution in the staining intensity of the nuclei, along with the presence of free chromatin material.

In one of the sections examined, there was a corpus luteum, but no corpora albicantia. In seven of the sections, there was a corpus luteum, with, in addition, several corpora albicantia, of varying sizes. In eight of the sections, there were corpora albicantia of varying sizes, but no corpus luteum. In one section, there were neither any corpora albicantia, nor a corpus luteum.

(2) Developing follicles.

Primordial follicles were present in almost all of the sections examined. These were all early primordial, consisting of an ovum surrounded by a layer of flattened cells. They varied in number from nil to 27.

In the whole series, there were no relatively more advanced follicles, while only one section showed the presence of one

fully developed follicle. This fully developed follicle was lined with thin theca interna, which did not show any proliferative change, or increased vascularity. In addition, there were traces of a granulosa layer round the antrum, and cumulus formation was present, the cumulus containing an ovum. Neither the theca nor the granulosa showed the presence of degenerative changes.

(3) Atretic follicles.

Atretic follicles formed the main point of interest, in the sections examined, as well as the presence or absence of a corpus luteum. These varied in size, some being small, while others were very large.

As in the early pregnancy series, both patent and collapsed varieties were present. Both patent and collapsed types were lined with theca interna, and in some, in addition granulosa tissue was also present.

The atretic follicles in the eighteen sections examined, showed the following features:-

The number of patent follicles varied from nil to 9.

The average number present was 2.9.

Only one collapsed follicle was present in the entire series.

(4) Areas of thecal change.

No areas of thecal change were present in any of the se ctions examined.

(5) Histological detail of the theca.

In three of the sections examined, there were no atretic follicles present, so the theca could not be studied.

Of the fifteen sections showing atretic follicles, the theca did not show the presence of degenerative changes in five cases. In the other ten, it showed degenerative changes of varying degree. In some it was slight, showing disappearance of some nuclei, with breaking up of other nuclei; in others it was more marked, showing in addition to the changes just described, diminution in the intensity of the staining of the nuclei, as well as the release

Figure 4. This shows a collapsed atretic follicle, the centre of which is filled with loose connective tissue. The theca is beginning to degenerate, and undergo replacement by hyaline material.

of free chromatin material.

In three of the sections, the follicles were lined with theca interna, there being no granulosa tissue present. In eleven of the sections, granulosa tissue was also present, lying internal to the theca interna. It was usually lying detached in the lumen of the follicle, although in some cases, it was 'in situ'.

The degree of degeneration of the granulosa varied as did the theca interna. In two sections, the theca adjacent to the lumen was being replaced by a layer of what appeared to be a hyaline material.* In one of these, the theca was degenerate, while in the other, it was not.

In one section, the theca next the lumen, was being replaced by a layer of fibrous tissue. In this case, the theca showed signs of degenerative change.x An analysis of the theca was then made, in order to establish the degree of activity that was present. Three criteria were used in this estimation. These were as follows:-

* See Figure 4.
x See Figure 5.

Figure 5. This shows a further stage in the fibrous replacement of the hypertrophic theca in an atretic follicle.

(1) Degree of proliferation.

(2) Vascularity.

(3) Combinations of these two.

The majority of the follicles showed theca that was some six layers thick, and this has been taken as a standard of normality. Increase in the number of layers above this figure, is assumed to indicate proliferation. Vascularity was estima ted as being the obvious increase in the number of blood vessels present in the theca interna.

The results of the analysis are as follows:-

Sections showing a degree of proliferation	4.
Sections showing vascularity	1.
Sections showing proliferation and vascularity	-.

(6) Areas of decidual change.

Five of the sections examined showed the presence of areas of decidual change. These were usually in the form of plaques, which were situated round the periphery of the section.

Summary of the findings in the two series of early pregnancy cases.

Allowing for the fact that both ovaries were not available for study in the majority of the cases, a corpus luteum was present in at least fifty per cent. of cases including the seven patients dying in early pregnancy, and in the eighteen specimens removed at laparotomy for ectopic pregnancy.

The presence of degenerative change of slight degree was seen in roughly half the corpora lutea which were present. In the other corpora lutea, degenerative changes were marked. Corpora albicantia were generally present, along with the corpora lutea, although in one of the cases obtained at laparotomy for ectopic pregnancy, there was a corpus luteum, without the presence of any corpora albicantia.

These corpora albicantia varied in both number and size, some of them being very large.

In ten of the entire series, corpora albicantia were present, without the presence of a corpus luteum.

Developing follicles were present in almost

all the sections examined. The primordial follicles varied in number from nil to thirty one, while in only one section was there a follicle which was of the relatively more advanced type.

Atretic follicles were present in the great majority of sections examined.

The patent variety were very much in the majority, although an occasional collapsed follicle was also seen.

The size and number of these patent follicles varied very greatly, the greatest number present in one section being nine.

All these follicles were lined with theca interna tissue, which in some cases was not degenerate. In the sections where degenerative changes were present, these varied from slight to well-marked.

In addition to the theca interna tissue, granulosa was also present in many of the sections. In general, this varied in degree of degeneration, as did the theca.

In the estimationof thecal activity, a

total of nine of the entire series, showed the presence of proliferative change, while only three showed the presence of increased vascularity.

From these figures, it will therefore be seen that thecal activity was seen in nine out of twenty five sections, i.e. in thirty six per cent.

(C) Late pregnancy cases.

A description of the forty-two cases dying in late pregnancy, will now be given, on the same lines as the description of the early pregnancy cases.

The duration of pregnancy at which death occurred, was as follows:-

Twenty-three cases died at	40	weeks pregnancy.
One case died at	39	weeks pregnancy.
Four cases died at	38	weeks pregnancy.
One case died at	37	weeks pregnancy.
Three cases died at	36	weeks pregnancy.
Two cases died at	35	weeks pregnancy.
One case died at	34	weeks pregnancy.
One case died at	33	weeks pregnancy.
Two cases died at	32	weeks pregnancy.
Three cases died at	30	weeks pregnancy.
One case died at	28	weeks pregnancy.

(1) The Corpus Luteum.

A corpus luteum was present in 28 of the 42 sections examined. In two of the sections, it was not degenerate, while in 17 of the sections, it showed slight degenerative changes. These consisted of disappearance of some nuclei, with breaking up of other nuclei. There was also some diminution in the staining intensity of the nuclei.

In 9 of the sections, there were marked degenerative changes. This was manifested by the disappearance of many nuclei, with breaking-up of a considerable number. There was a marked diminution in the intensity of the staining of the nuclei, and free chromatin material was evident.

In all the corpora lutea, the central lumen was filled with what appeared to be a hyaline material. In a few cases, this was partly replaced by fibrous tissue.

In 20 of the sections examined, the corpus luteum was large. In one, it was of moderate size, whereas in 7 sections, it was small.

In 24 of the sections, the corpus luteum was accompanied by the presence of corpora albicantia, of varying sizes, some being very large.

In 4 of the sections, a corpus luteum was present, without any corpora albicantia.

In 14 of the sections, corpora albicantia were present, but no corpus luteum was present.

(2) Developing follicles.

Primordial follicles were present in the great majority of the sections examined. The number present varied very greatly, as although a few sections did not contain any primordial follicles, one section contained as many as 295.

The average number of primordial follicles present was 34.

Primordial follicles, which were further advanced, were present in 19 of the sections. The number present varied from nil to 7.

The average number present was 2.

Fully-developed follicles were present in 15 of the sections examined. The number present varied from nil to six.

The average number present was 0.9

(3) Atretic follicles.

Both patent and collapsed varieties were present, in varying numbers, in all the sections examined.

The number of patent follicles varied from nil to 17.

The average number present was 4.1.

The number of collapsed follicles varied from nil to 11.

The average number present was 2.7.

(4) Areas of thecal change.

17 of the sections examined showed the presence of areas of thecal change.

The number of such areas present, varied from nil to 10.

The average number present was 1.6.

(5) Histological detail of the theca.

In 25 of the sections examined, the theca did not show any evidence of degeneration. In the other sections, it showed degenerative changes, of varying degree. The changes varied from slight degenerative changes (disappearance

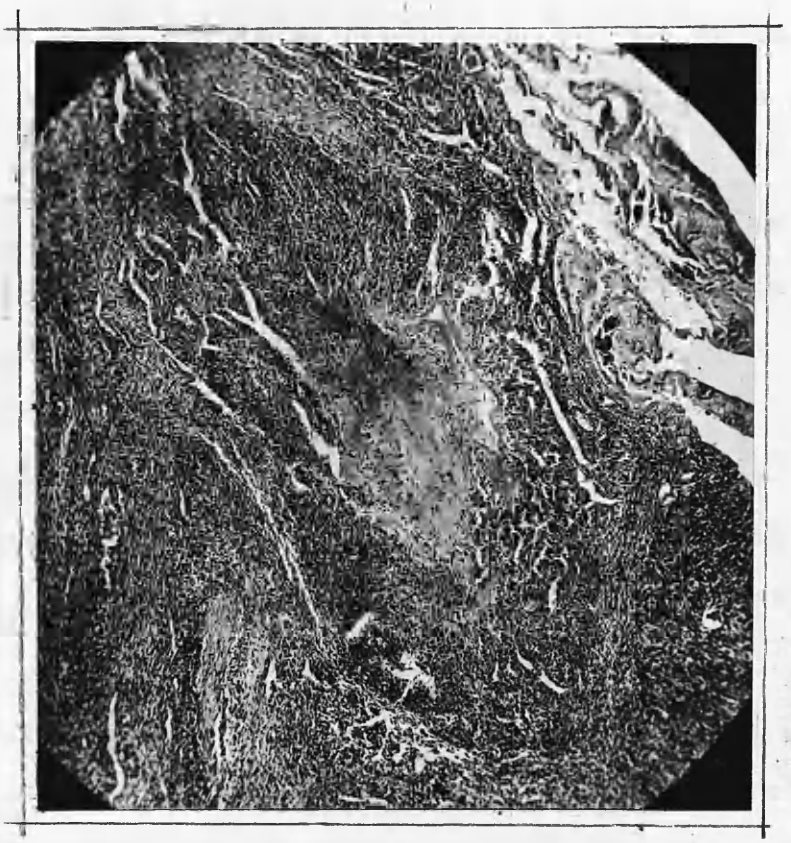

Figure 6. This shows an appearance sometimes seen in the degenerating thecal tissue. A small type of corpus albicans of wavy hyaline material can be seen forming on one aspect.

of some nuclei, with breaking-up of other nuclei) to more marked degenerative changes (in addition to the changes just mentioned, diminution in the intensity of the staining of the nuclei, as well as the release of chromatin material).

In 18 of the sections examined, granulosa tissue was present, internal to the theca interna. It was usually lying detached, lying in the lumen, in the case of the atretic patent follicles. In the collapsed follicles, the granulosa was usually lying in apposition to the theca.

The degree of degeneration of the granulosa, varied as did the theca interna.

In the sections showing a greater degree of degeneration, a marked feature was the replacement of the theca interna or granulosa, surrounding the lumen of the follicle (in the patent variety) or what remained of the lumen (in the collapsed variety) with a layer of hyaline material, or even fibrous tissue. This layer varied from a thin strip,* to a broad band, but in two sections, there were patent atretic

* See Figure 6.

follicles, where the theca interna had been entirely replaced by hyaline material. In one section, there was an additional point of interest, in that the theca interna had been almost entirely replaced with hyaline material, and yet the theca interna that was still identifiable, did not show any degenerative changes. An analysis of the theca interna was made, in an effort to estimate the degree of activity. The following criteria were used:-

(1) Degree of proliferation.

(2) Vascularity.

(3) Combinations of these two.

Degree of proliferation was judged by the theca interna being more than six layers thick. The average thickness, where proliferation was not present, varied from three to four layers.

Vascularity was judged to be an obvious increase in the number of blood vessels present in the theca interna.

The results of the analysis are as follows:-

(a) <u>The section showing the greatest activity, had the following details:-</u>

A corpus luteum was present; it was large and degenerate. Several corpora albicantia were also present.

Primordial follicles numbered	295
More advanced follicles numbered	6
Fully developed follicles numbered	2
Patent atretic follicles numbered	2
Collapsed atretic follicles numbered	5
Areas of theca change numbered	4
Histological detail of the theca	Proliferation only.

(b) <u>The second most active section showed</u>:-

No corpus luteum was present, but there were several corpora albicantia of varying size.

Primordial follicles numbered	146
More advanced follicles numbered	7
Fully developed follicles numbered	2
Patent atretic follicles numbered	2
Collapsed atretic follicles numbered	8
Areas of thecal change numbered	8
Histological detail of the theca	Proliferation vascularity.

(c) The third most active section showed:-

A large corpus luteum was present, which showed slight degenerative changes.

Several corpora albicantia were also present.

Primordial follicles numbered	3
More advanced follicles numbered	3
Fully developed follicles numbered	3
Patent atretic follicles numbered	4
Collapsed atretic follicles numbered	7
Areas of thecal change numbered	1
Histological detail of the theca	Proliferation vascularity.

(d) The fourth most active section showed:-

A small corpus luteum was present. This showed only slight degenerative changes. No corpora albicantia were present.

Primordial follicles numbered	86
More advanced follicles numbered	5
Fully developed follicles numbered	3
Patent atretic follicles numbered	4
Collapsed atretic follicles numbered	11
Areas of thecal change numbered	1
Histological detail of the theca	Proliferation vascularity.

(e) The fifth most active section showed:-

A large, degenerate, corpus luteum was present. In addition, several corpora albicantia were present, of varying size.

Primordial follicles numbered	35
More advanced follicles numbered	5
Fully developed follicles numbered	nil.
Patent atretic follicles numbered	16
Collapsed atretic follicles numbered	1
Areas of thecal change numbered	nil.
Histological detail of the theca	Proliferation only.

(f) The sixth most active section showed:-

A large corpus luteum was present, which showed only slight degenerative changes. Several corpora albicantia were also present.

Primordial follicles numbered	67
More advanced follicles numbered	6
Fully developed follicles numbered	6
Patent atretic follicles numbered	4
Collapsed atretic follicles numbered	3
Areas of thecal change numbered	10
Histological detail of the theca	Proliferation vascularity.

At this stage, it was decided to see if there was any relation between the activity of the ovary, and the clinical condition of the patient. The subdivision used, was the rather rough one of cases of toxaemia, and those who did not show any evidence of toxaemia. The results of this were as follows:- Of the 42 sections examined, 14 were taken from cases who had had manifestations of toxaemia, while 28 were taken from cases who did not show any such manifestations.

In the case of the 6 sections showing the greatest activity, the causes of death were as follows:-

Arranged in order of activity.

(1) Eclampsia.

(2) Haemorrhage associated with placenta praevia.

(3) Eclampsia.

(4) Haemorrhage and shock associated with retained placenta.

(5) Rheumatic carditis and cerebral thrombosis.

(6) Eclampsia.

The conclusions that are evident are

therefore:-

Out of the whole group, the number of cases not showing toxaemia was just twice the number who had toxaemia.

Of the six showing the greatest activity, the number of toxaemia cases was exactly the same as the number of cases not showing toxaemia.

(D). A further group of cases of late pregnancy, mostly obtained at laparotomy for Caesarean Section.

A total of twenty sections was obtained, being taken at laparotomy for Casearean Section.

The duration of pregnancy at which the specimen was obtained, was as follows:-

Sixteen cases at 40 weeks pregnancy.

Two cases at 38 weeks pregnancy.

Two cases at 35 weeks pregnancy.

Unfortunately, in all these cases, only specimens from one ovary were available for study.

(1) The Corpus Luteum.

A corpus luteum was present in five of the twenty sections examined. In four of the sections, it was not degenerate, while in the other one, it showed slight degenerative changes. These consisted of disappearance of some nuclei, with some diminution in the staining intensity of the nuclei.

In all the corpora lutea, the central

lumen was filled with what appeared to be a hyaline material. In two of the cases, this was partly replaced by fibrous tissue.

In all five of the sections examined, the corpus luteum was large.

In three of the sections, the corpus luteum was accompanied by the presence of corpora albicantia, of varying sizes, some being very large.

In two of the sections, a corpus luteum was present, without any corpora albicantia.

In twelve of the sections, corpora albicantia were present, but no corpus luteum was present.

(2) Developing follicles.

Primordial follicles were present in the great majority of the sections examined. Once again, the number present varied very greatly, the variation being from none to as many as one hundred and ninety-six.

The average number of primordial follicles present was 20.5.

Primordial follicles, which were further

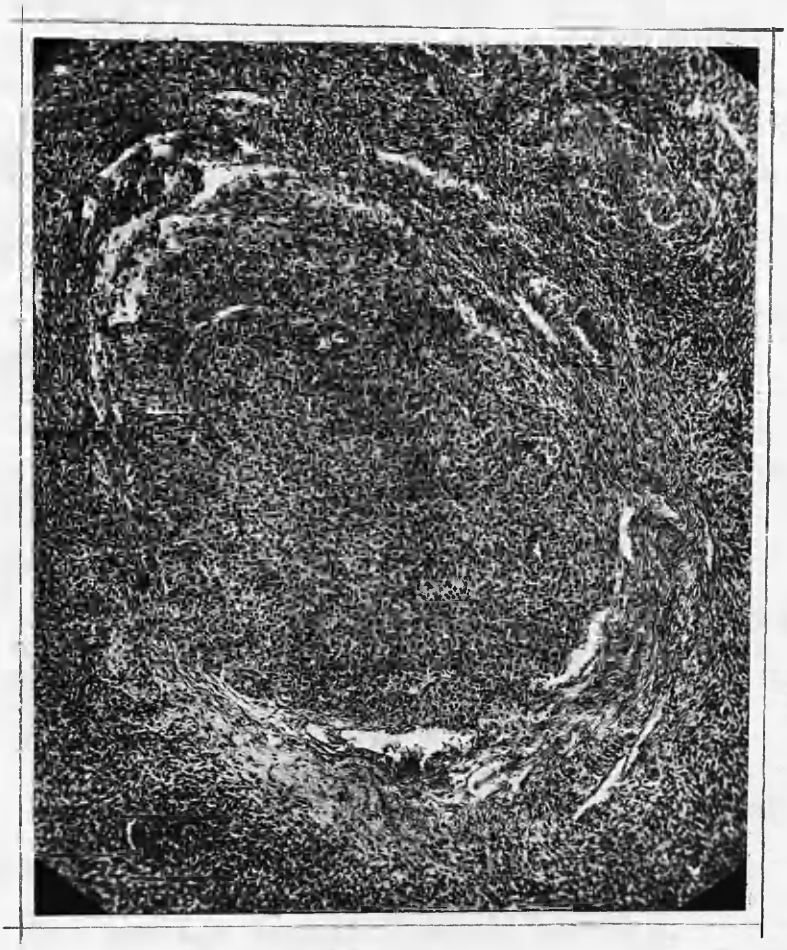

Figure 7. This shows a solid nodule of thecal tissue. Whether these arise from developing follicles or mainly from the interstitial tissue is not clear.

advanced, were present in eight of the sections.

The number present varied from nil to four.

The average number present was 0.65.

Fully-developed follicles were present in two of the sections examined. The number present varied from nil to one.

The average number present was 0.1.

(3) Atretic follicles.

Both patent and collapsed varieties were present, in varying numbers, in seventeen of the twenty sections examined.

The number of patent follicles varied from nil to seven.

The average number present was 1.7.

The number of collapsed follicles varied from nil to five.

The average number present was 1.2.

(4) Areas of thecal change.

Nine of the sections examined showed the presence of areas of thecal change.*

The number of such areas present, varied from nil to three.

The average number present was 0.75.

* See Figure 7.

(5) Histological detail of the theca.

Only in one of the sections examined, did the theca show any evidence of degeneration. This was shown by the absence of some nuclei, and breaking up of other nuclei. There was also diminution in the staining intensity of the nuclei.

In eleven of the sections examined, granulosa tissue was present, lying internal to the theca interna. In some cases, it was 'in situ', while in others, it was lying loose in the lumen of the follicle.

The degree of degeneration of the granulosa varied, as did the theca interna. In some of the follicles, there was a layer of hyaline material, replacing the theca interna. This layer was situated next to the lumen of the follicle, and in some cases, there was also a layer of fibrous tissue, internal to the hyaline layer. The appearance of the hyaline layer did not seem to be necessarily related to the presence of degenerative changes in the theca.

An analysis of the theca interna was then made, in order to estimate the degree of activity.

As before, the following criteria were used:-

(1) Degree of proliferation.

(2) Vascularity.

(3) Combination of these two.

A degree of proliferation was said to be present, when the cells were some six layers thick. This was present to a much greater degree in some of the sections, and this was commented upon.

Vascularity was judged to be an obvious increase in the number of blood vessels present in the theca interna.

The results of the analysis are as follows:-

Sections showing degree of proliferation	16.
Sections showing vascularity	4.
Sections showing proliferation and vascularity	4.

In the above list, two of the sections showed marked proliferation of the theca interna. One of them showed, in addition, an increase in vascularity, while the other did not. In this latter section, the theca showed proliferation to a most marked degree, with the presence of a

very large area of thecal change.

(6) Areas of decidual change.

Twelve of the sections showed the presence of areas of decidual change. These were usually in the form of plaque areas, and were almost always seen round the periphery of the section.

Once again, as ovarian activity varied from section to section, it was decided to ascertain the features of the slides showing the greatest activity - in this case four.

The usual criteria were adopted, viz.,

(1) The presence and condition of a corpus luteum, or corpora albicantia.

(2) A count of the developing follicles, in various stages of maturation.

(3) A count of the types of follicles which have gone on to atresia.

(4) A count of the areas of thecal change.

(5) The above results related to the activity of the theca.

(a) The section showing the greatest activity had the following details:-

No corpus luteum was present, but there

were several corpora albicantia, of varying size, some of them being very large.

Primordial follicles numbered	4.
More advanced follicles numbered	4.
Fully developed follicles numbered	1.
Patent atretic follicles numbered	7.
Collapsed atretic follicles numbered	1.
Areas of thecal change numbered	-
Histological detail of the theca	Proliferation. Vascularity.

(b) The second most active section showed:-

No corpus luteum was present, but there were several corpora albicantia of varying size.

Primordial follicles numbered	19.
More advanced follicles numbered	-.
Fully developed follicles numbered	1.
Patent atretic follicles numbered	2.
Collapsed atretic follicles numbered	1.
Areas of thecal change numbered	3.
Histological detail of the theca	Proliferation only.

(c) The third most active section showed:-

No corpus luteum was present, but there were

the number of toxaemic cases was exactly the same as the number of cases not showing toxaemia.

(D). The specimen obtained from a woman dying three months after delivery.

In this case pregnancy and labour had been normal, apart from the fact that the patient had been suffering from cardiac disease. The cause of death was heart failure.

The specimen available was only taken from one ovary.

(1) The Corpus Luteum.

No corpus luteum was present in the section examined, but there were several corpora albicantia of varying size.

(2) Developing follicles.

Only two primordial follicles were seen, both of them being surrounded with a single layer of flattened cells, and containing an ovum.

There was also one fully-developed follicle, which was lined with theca interna. Granulosa tissue was also present, and formed a cumulus, which contained an ovum.

Neither the theca nor the granulosa showed degenerative changes, although the theca was thin, and relatively non-vascular.

(3) Atretic follicles.

There were no atretic follicles present in the section examined.

(4) Histological detail of the theca.

The section examined, did not show proliferation of any marked degree, or any increased vascular change.

(5) Areas of thecal change.

There were no areas of thecal change present.

(6) Areas of decidual change.

There were no areas of decidual change present.

Summary of the findings obtained in the two series of cases obtained in late pregnancy.

The total number of cases studied was sixty two, which was made up as follows:-

(1) A group of forty two cases, obtained at post-mortem examination on cases dying in late pregnancy.

(2) A second group of twenty specimens obtained from patients undergoing laparotomy for Caesarean Section.

In the first series, a corpus luteum was present in exactly sixty six and two thirds per cent. of cases. It showed little or no degeneration in s xty eight per cent. of these cases, and marked degeneration in thirty two per cent.

In the second group a corpus luteum was present in twenty five per cent. of cases.

It was not degenerate in eighty per cent. of the cases, and showed only slight degenerative changes in the remaining twenty per cent.

Primordial follicles were present in almost every section in both the groups. The average

nnumber per section, in the first group, was 34; while in the second group it was 20.5.

The average numbers of further advanced follicles were as follows:-

(1) Two in the first group.

(2) 0.65 in the second group.

In the first group, the average number of fully developed follicles was 0.9, while in the second group it was 0.1.

Atretic follicles were present in the great majority of sections in both groups. Both patent and collapsed varieties were present, the average numbers present being:-

Patent: First group, 4.1, second group 1.7.

Collapsed: First group, 2.7, second group 1.2.

The average number of thecal areas was as follows:-

First group, 1.6, second group, 0.75.

The activity of the ovaries in both groups, was judged by an analysis of the theca interna. This was studied, both in the atretic follicles, and in the areas of thecal change.

As stated previously, the criteria of thecal activity were:-

(1) A degree of proliferation.

(2) An increase of vascularity.

(3) Combinations of these two features.

The results obtained were as follows:-

In the first group -

Total number showing a degree of proliferation	12.
Total number showing increased vascularity	3.
Total number showing both proliferation and vascularity	16.

In the second group -

Total number showing a degree of proliferation	16.
Total number showing increased vascularity	4.
Total number showing both proliferation and vascularity	4.

Degenerative changes were present as follows:-

In the first group. In seven of the sections examined, i.e. in 16.6 per cent.

In the second group. In one of the sections examined, i.e. in 5 per cent.

Areas of decidual change were present in twenty four sections of the first group, and

in twelve sections of the second group.

Experimental Work.

(1) From the results obtained by a study of the sections taken from cases at different stages of pregnancy, it seems obvious that there is ovarian activity in both early and late pregnancy.

(2) However, as term approaches, it is seen that the most marked feature is an increase in follicular activity and maturation, with the production of large masses of internal theca. These changes raise many questions of endocrine control of ovarian activity and hormone production. Only one of these problems has been tackled in the present thesis.

(3) Since chorionic gonadotrophin does not appear to have much influence on the human ovary, the findings would suggest that some other gonadotrophin is present during pregnancy, and its activity increases, as term approaches.

It was therefore decided to test this hypothesis by animal experiment. On the assumption that these gonadotrophins are excreted in the urine, extracts were prepared from the

urine of patients at various stages of pregnancy. Care was taken to avoid any patient suffering from toxaemia, in whom the hormone levels might be altered, as stated before, in the introduction.

Accordingly, the selection of patients was confined to those suffering from cardiac disease, who had been admitted for rest; contracted pelvis; anaemia; rest over suppressed menstrual periods, etc.

In both the early and the late pregnancies, extracts were made, using both kaolin and benzoic acid.

A synopsis of the methods used, in the preparation of these extracts is now given:-

Kaolin Method.

A litre of pregnancy urine is taken, and 20 per cent. hydrochloric acid is added to it, until the pH falls to 3.94.

100 mls. of a 20 per cent. suspension of Kaolin in water, is then added, and the mixture is stirred. The kaolin is then removed by being centrifuged. Water is then added to the Kaolin, in order to make a suspension. To this

suspension is now added 40 per cent. sodium hydroxide, until the pH rises to 11.3, and the mixture is allowed to stand for 20 minutes. (Loraine and Brown have shown that in the Kaolin method, all gonadotrophins can be eluted by raising the pH to 11.3).

The kaolin is therefore removed by being centrifuged, and is now discarded.

To the supernatant fluid, is now added, 30 ml. of calcium phosphate gel, and the pH reduced to 8. This mixture is stirred for 20 minutes, and then centrifuged. The supernatant fluid is then discarded, and the gonadotrophins adsorbed on the gel, is eluted with 40 ml. of .02 molar trisodium phosphate. To the eluate is added 20 per cent. hydrochloric acid, until the pH falls to 5.5.

Five volumes of acetone are now added, to precipitate the crude hormone.

The resulting mixture is then left in the refrigerator for 12-16 hours, overnight.

The crude hormonal precipitate is then removed by being centrifuged. It is finally washed in acetone, and left to dry in the air.

Benzoic Acid Method.

Two litres of pregnancy urine are taken, and 20 per cent. hydrochloric acid is added to it, until the pH falls to 3.92.

Then 400 ml. of 20 per cent. benzoic acid in acetone, is added to the urine, with frequent stirring, and the mixture is left in the refrigerator for 12-16 hours, overnight.

The supernatant fluid is then removed, and to the benzoic acid precipitate, 200 ml. of acetone are added. The mixture is then centrifuged.

The residue is now taken up in 120 ml. of acetone, and the mixture again centrifuged.

The residue is now treated with water (pH 9.9) and the mixture centrifuged.

The supernatant fluid is now decanted, and the residue washed with a further 80 ml. of water (pH 9.9).

The mixture is again centrifuged, and the supernatants are combined by mixing. The pH is then brought down to 4.5 by the addition of 20 per cent. hydrochloric acid.

Five volumes of acetone are now added, and

corrosive. They were embedded in paraffin, and sections were cut at a thickness of 6μ They were stained with haemalum and eosin.

In each case, the rats were injected into the peritoneal cavity, the left and right sides being used alternately. They were all killed by inhaling chloroform.

When studying the sections of ovaries, comparison was made with a set of control ovaries taken from female rats aged 21-25 days, who had not received any hormonal injections.

These experimental sections were not examined in the same way as the human specimens, but were examined purely with a view to the estimation of activity.

The presence of primordial follicles was noted, although an accurate count was not made. Rather, an impression was formed of the sections containing a greater number, as compared to those that contained relatively less.

Primordial follicles, in appearance, were very similar to those appearing in the human sections. They were either in the very early

the mixture left overnight in the refrigerator.

Next day, the precipitate is removed by being centrifuged. It is washed three times in acetone, and left to dry in the air.

The preparations of early pregnancy urine were standardised by Dr. S.M. Walker using the rat prostate assay method for L.H.

The animals chosen were all 21-day female rats, and all of them were given 1 ml. (11 units) of the hormone preparation.

The rats injected with early pregnancy urine, were divided into two groups, one of the groups being injected with the kaolin extract of the hormone, and the other group with the benzoic-acid extract.

Each group was then divided into six groups of six rats, these subdivisions being associated with the different times when the rats were killed. After being injected, the times were noted carefully, and the rats were killed at intervals of 12, 24, 48, 72, 96, and 120 hours. In all cases, the ovaries were removed immediately after death, and were fixed in formol-

form, consisting of an ovum surrounded by a single layer of flattened cells, or were of the further advanced type, where the ovum was surrounded by several layers of granulosa cells, with, often the appearance of a developing antrum.

Compared to the human specimens, there were relatively many more of the further developed variety, which were present in all sections of both the benzoic acid, and the kaolin series. In none of these was there any evidence of any degenerative changes.

In the benzoic acid series, there were a relatively great number of primordial follicles present in one of the specimens killed after 12 hours. This same appearance presented itself also, in one of the specimens killed after 48 hours; in one of the specimens killed after 72 hours; in three of the specimens killed after 96 hours, and in one of the specimens killed after 120 hours.

The presence of many primordial follicles was also seen in the kaolin series, although the distribution was rather different to the benzoic

acid series.

Many primordial follicles were seen in two of the specimens killed after 12 hours; in one of the specimens killed after 72 hours; in two of the specimens killed after 96 hours, and in all six of the specimens killed after 120 hours.

In addition to the further developed type of primordial follicle, many sections contained one fully developed follicle. These follicles were similar in appearance to those found in the human sections.

In addition to the above description, all the sections examined, in both series, contained many atretic follicles. These were lined with theca interna tissue. In some of the fully developed follicles, the remains of the cumulus was present, whereas in others only a thin strip of granulosa remained around part of the antrum. Conversely, some other follicles appeared to consist almost entirely of a large mass of granulosa, from which all evidence of an ovum had disappeared.

A search was then made for the presence of

a corpus luteum. In the benzoic acid series, the presence of a small corpus luteum was first noticed in one of the specimens killed after 48 hours. There was also an early corpus luteum developing from an atretic follicle, in another specimen killed after 48 hours.

In the sections taken from specimens killed after 72 hours, corpora lutea were seen in four cases. In each instance, the corpora lutea were developing from atretic follicles.

Of the sections taken from specimens killed after 96 hours, corpora lutea, developing from atretic follicles, were present in two cases. In the group killed after 120 hours, one large corpus luteum was seen in one section.

Multiple corpora lutea, both large and small, were seen in two of the specimens killed after 96 hours. In one of them, there were as many as 6 large corpora lutea.

There were two large, and one small, corpora lutea, in one of the specimens, killed after 120 hours.

In contrast, in the kaolin series, no corpus

luteum was present, in any of the series which had been killed after 48 hours. In the series killed after 72 hours, there was one small corpus luteum in one section. In another section, there were four large corpora lutea.

In one example in the series killed after 96 hours, there were six early corpora lutea, all of which were developing in atretic follicles.

In one of the series killed after 120 hours, there was a developing corpus luteum. This was especially interesting in that it seemed to be developing from the wall of a fully developed follicle. In addition to this, there was also one large corpus luteum, present in the same section.

Multiple corpora lutea were present in one of the series killed after 96 hours (two large and one small); and in one of the series killed after 120 hours (two large and one small).

In the rat ovary, unlike the human, the interstitial tissue consists of cells which resemble the theca interna of the human ovary.

An estimation of stromal activity was then made, on the basis of the density of the tissue. Four stages of density were noted, and were designated as +, ++, +++, and ++++.

In the benzoic acid series, the results were as follows:-

One section showed +.

Fourteen sections showed ++.

Twelve sections showed +++.

Five sections showed ++++.

In the kaolin series, the results were as follows:-

One section showed +.

Eighteen sections showed ++.

Twelve sections showed +++.

Five sections showed ++++.

In no case, in any of the sections, was there any evidence of degenerative changes in the theca interna or granulosa.

The rats injected with late pregnancy urine, were divided into three groups, depending on whether the benzoic acid or the kaolin extract was used. One group was injected with benzoic acid

extract, and the other two with kaolin extracts. Each group was subdivided into six groups as before, these sub groups being killed at the same time intervals used for the rats injected with early pregnancy urine. The preparations were standardised, as in the early pregnancy series, and the same dosage was employed. The rats were all injected with 1 ml. using the same technique as before. The sections of ovaries were prepared as stated previously. The appearance of the primordial follicles, was just the same as in the specimens injected with early pregnancy urine.

In the benzoic acid series, there were a relatively great number of primordial follicles present in two of the specimens killed after 48 hours; this appearance was also seen in four out of the six specimens killed after 73 hours; and in one of the specimens killed after 96 hours. Unlike the specimens in the early pregnancy series, the specimens killed after 120 hours did not show any increase in the number of primordial follicles.

In the kaolin series, increased numbers of follicles were seen in two of the specimens killed

after 24 hours; in three specimens killed after 48 hours; in four of the specimens killed after 72 hours; in seven specimens killed after 96 hours, and in five of the specimens killed after 120 hours. These figures are the combined result of the two series injected with kaolin extract.

The appearance and number of fully developed follicles was almost the same as in the early pregnancy series. Similarly, the number and description of the atretic follicles, is the same as in the early pregnancy series.

A search was then made for the presence of a corpus luteum, as in the early pregnancy series. In contrast to the corpora lutea already described in that series, there was a marked difference, both in the number of corpora lutea, and also as regards the times when they appeared.

In the benzoic acid series only one small corpus luteum was found. This appeared in one of the specimens which were killed after 72 hours.

In the kaolin series, one section showed a large corpus luteum. This was present in one of the specimens killed after 120 hours. In the

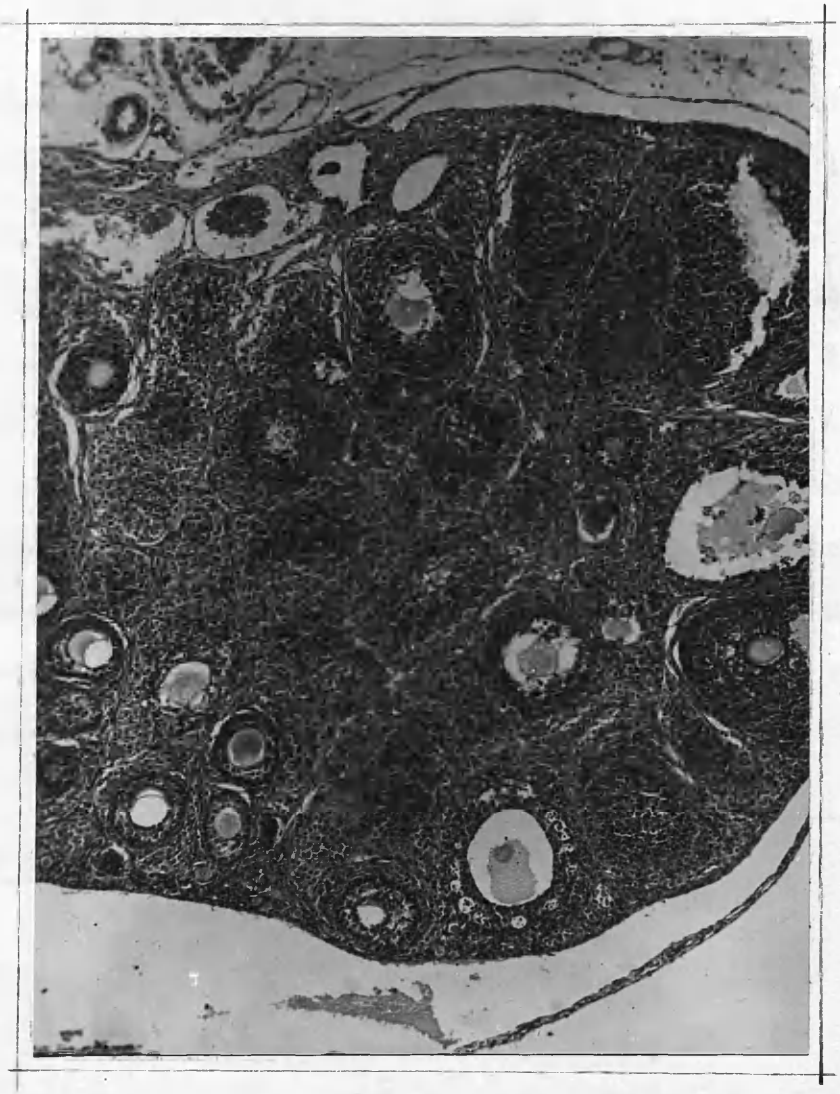

Figure 8. Ovary from 23 day old rat, 48 hours following injection with an extract of late pregnancy urine. The medulla of the ovary is now filled by masses of interstitial cells which are actively proliferating.

other kaolin series, there was only one small, developing corpus luteum. This was present in one of the specimens killed after 120 hours.

Apart from these three corpora lutea, no others were present at all, in any of these three series.

An estimation of stromal activity was then made, in the same way as for the early pregnancy specimens. The four stages of density were noted in the benzoic acid series, and also in the two kaolin series.

In the benzoic acid series, the results were as follows:-

Ten sections showed ++.

Thirteen sections showed +++. *

Seven sections showed ++++.

There was no evidence of degeneration in any of the sections examined.

In the kaolin series, the results were as follows:-

In the first series -

Eight sections showed ++.

Twenty sections showed +++.

* See Figure 8.

Six sections showed ++++.

In the second series -

One section showed +.

Eleven sections showed ++.

Twenty sections showed +++.

Four sections showed ++++.

In none of the kaolin series, was there any evidence of degenerative changes seen in the theca interna.

The control series was then examined, to determine what changes, if any, had occurred, without any of the hormone injections. This series consisted of a total of ten female rats, of which two were killed aged 21 days; two were killed aged 22 days; two were killed aged 23 days; two aged 23 days, and two aged 24 days. The general appearance of these sections was similar in many ways, to the series which had been injected with hormone. Simple primordial follicles were present in almost all the sections, and these consisted of an ovum surrounded by a layer of flattened cells. In addition, there were again an increase in the number of follicles, showing more advanced

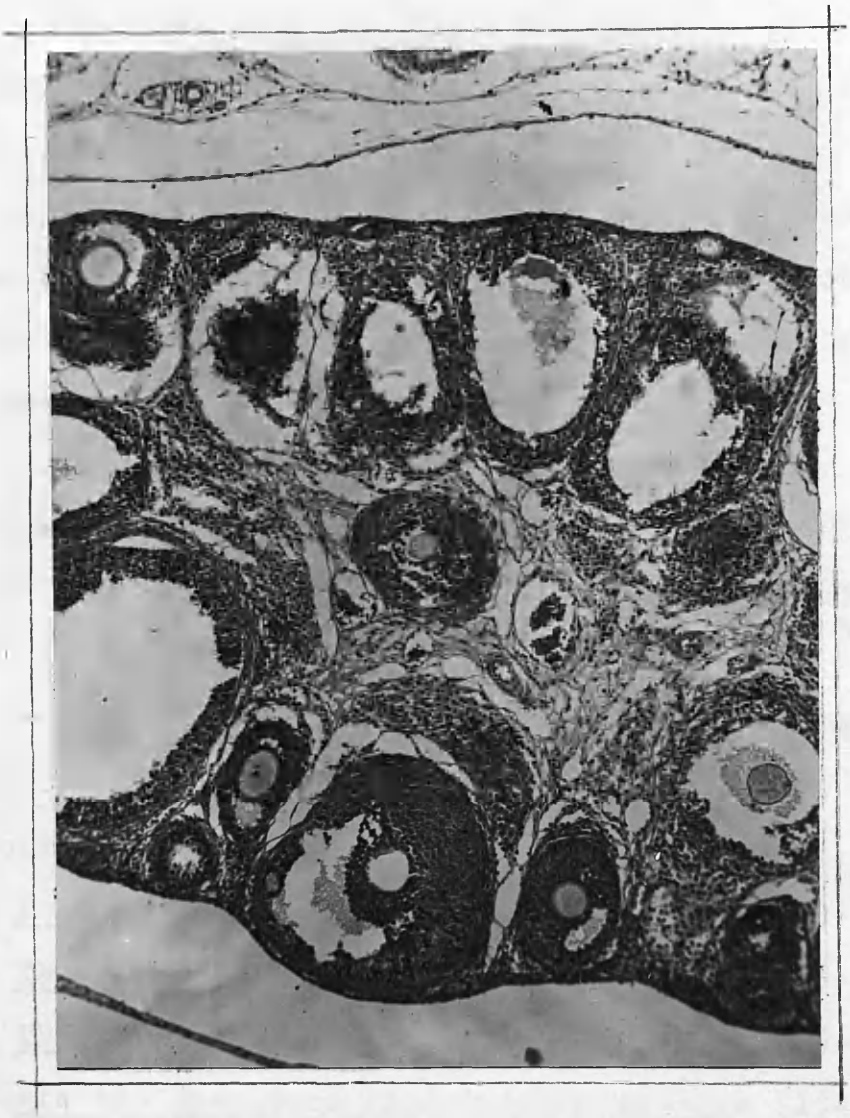

Figure 9. Ovary from 23 day old rat. Interstitial cells are only beginning to make their appearance, and the medulla of the ovary is composed of loose connective tissue.

change. There were also some fully developed follicles, and many atretic follicles, all similar in appearance to the hormone-injected series. A study of the theca interna present, as the interstitial tissue, however, showed a marked difference.

The theca interna did not show any degenerative changes, as in the hormone injected series, but there was a very obvious difference in the density of the tissue.

The same four stages of density were used, as described previously, and the results were as follows:-

The two specimens killed after 21 days (1)+ (2)++.

The two specimens killed after 22 days (1)+ (2)+.

The two specimens killed after 23 days (1)+ (2)++. *

The two specimens killed after 24 days (1)+ (2)++.

The two specimens killed after 25 days (1)+ (2)++.

Conclusions to be drawn from the experimental work.

(1) From a study of the interstitial tissue, seen in the injected series, as compared to the control series, it seems probable that there is a

* See Figure 9.

hormone present in the urine taken from early pregnancy cases. This hormone causes the proliferation of theca interna, its effect being seen as early as twelve hours after injection.

(2) The same effect is seen in the cases injected with late pregnancy urine, as there too, thecal proliferation was seen as early as twelve hours after injection.

(3) A comparison between the sections from specimens injected with early pregnancy urine, and those injected with late pregnancy urine, shows that in the case of early pregnancy urine, there is also a hormone that plays a part in the development of corpora lutea.

This hormone seems to be present in greater concentration in the benzoic acid series, when it is considered that corpora lutea appear earlier, and are more numerous, than in the series injected with the kaolin extract.

(4) Whatever this hormone may be, it seems to be missing from the urine taken from late pregnancy cases, as in the specimens injected with late pregnancy urine, a total of only three

corpora lutea were seen. In the benzoic acid series, as stated previously, only one small corpus luteum appeared. It was present in one of the specimens killed after 72 hours.

In the two kaolin series, there were only two corpora lutea present. One was in each series, and both appeared in animals killed after 120 hours.

(5) From the fact that corpora lutea were few in number, and only appeared at a late date, in animals injected with extracts of late pregnancy urine, another conclusion may be drawn. It seems possible that in late pregnancy the hormone present does not itself cause the formation of corpora lutea, but rather that it sets in motion a train of events, which stimulates the animal's own pituitary to produce luteinising hormone.

Summary of the findings obtained in the experimental work.

The ovaries were obtained from a series of control rats, a total of ten being used. Of these, two were killed, aged twenty one days; two were killed aged 22 days; two were killed aged twenty three days; two were killed aged twenty four days, and two were killed aged twenty five days.

The histological appearance of these ovaries was compared with two series of rats, injected with a hormonal extract, obtained from (a) human early pregnancy urine; (b) human late pregnancy urine. In both the early and the late pregnancy urines, hormonal extracts were prepared by two methods (1) Kaolin extract; (2) Benzoic acid extract.

Early pregnancy extracts.

The rats injected with early pregnancy urine were divided into two groups, one of them being injected with the kaolin extract of the hormone, and the other with the benzoic acid extract. After injection the rats were killed at intervals of 12, 24, 48, 72, 96

and 120 hours.

An estimation of ovarian activity was then made by a study of -

(a) Primordial follicles.

(b) Atretic follicles.

(c) Corpora lutea.

It was observed, that in distinction to the human ovary, there were relatively many more of the further developed type of primordial follicles. These were present in all the sections of both the benzoic acid and the kaolin series.

Many primordial follicles were seen, in various sections, taken from specimens killed after the different time intervals, in both the benzoic acid, and the kaolin series.

Many sections were found to contain one fully developed follicle. These were similar in appearance to those found in the sections of human ovaries. Allthe sections examined, in both series, contained many atretic follicles, These were all lined with thecainterna tissue, while granulosa was also present in some.

Neither the theca nor the granulosa showed evidence of degeneration. A search for corpora lutea, showed that one appeared within forty eight h ours of hormone injection. Further corpora lutea were seen in specimens killed at the different times, as described, and inthe cases toward the end of the series, multiple corpora lutea were seen.

Stromal activity was estimated on the density of the interstitial tissue, which consisted of cells similar to those forming the theca interna in the human ovaries. In both the benzoic acid and the kaolin series, stromal activity was present in all the sections, even in those obtained after 12 hours.

Latr pregnancy extracts.

The rats injected with late pregnancy urine were again divided into two groups, one being injected with benzoic acid extract, and the other with kaolin extract. The appearance and occurrence of primordial follicles was the same as in the early pregnancy urine series.

The appearance and number of fully

developed follicles, and atretic follicles, was the same as in the early pregnancy urine group.

With regard to the occurrence and number of corpora lutea, there was a marked difference, as compared to the series injected with early pregnancy urine. Only one corpus luteum occurred in the benzoic acid series, and it was seen after seventy two hours.

Two kaolin preparations were available for study. In both instances the results were similar. Only one corpus luteum was found in each. In both cases, it occurred after one hundred and twenty hours.

Stromal activity was found to be roughly the same as in the early pregnancy urine series.

Finally, an examination of the control series, showed a marked difference, with comparison to the series injected with hormonal extract. In the control series, stromal activity was very slight indeed, in all the sections examined.

The conclusions reached were:-

(1) A hormone was present in both the early and the late pregnancy urines which caused

proliferation of the interstitial cells.

(2) In pregnancy urine, there was also a hormone which played a part in the development of corpora lutea.

This hormone seemed to be present in greater concentration in the benzoic acid extract.

(3) Although corpora lutea were found in the animals injected with late pregnancy extracts they were infrequent and only formed one hundred and twenty hours after injection. Two possibilities arise:- a) The hormone responsible for corpus luteum formation was present in low concentration or b) the luteinising hormone was absent and any corpus luteum formation was due to the stimulation of the animal's own pituitary. The second of these hypotheses seems the more likely since most studies have shown that the activity of these hormones follows the "all or none" rule and have little relationship to time per se.

A clinical investigation into ovarian activity in pregnancy as manifested by the vaginal epithelium.

It is a well-known fact that there is a progressive increase in the urinary oestrogen, during pregnancy. This increase rises to its peak just before the onset of parturition, this rise being accompanied by a parallel rise in the blood oestrogen concentration.

After delivery the oestrogen levels rapidly decline.

In the work embodied in the thesis, it has been suggested that ovarian activity continues during pregnancy, being more marked in the late stages of pregnancy. It was considered that the rising oestrogen levels might have their origin in the ovary and the pituitary, rather than in the placenta.

It was then decided to observe if the changes in the vaginal epithelium agreed with the findings as found in the ovarian studies, i.e. increasing activity in late pregnancy, as compared to early pregnancy, and a decline in

ovarian activity following parturition. The way in which this was done was by means of an examination of vaginal smears taken at different stages of pregnancy, and also during the puerperium.

The smears were examined after being treated in two different ways, these ways being:-

(1) After exposure to iodine vapour.

(2) After being stained according to the method of Papanicolaou.

In the first method the vaginal smears were taken and were at once rubbed on to clean glass slides. They were then placed in a jar, in the base of which were fresh iodine crystals, and the jar covered. After five minutes exposure to the iodine fumes, the slides were then examined for the presence of glycogen. This was considered to be present when the cells were found to have undergone a change in colour, to a deep mahogany brown. In the cases where there was no change in colour, or where the cells stained a light yellow colour, glycogen was said to be absent.

The patients from whom the smears were taken were divided into four groups as follows:-

(1) Early antenatal cases, up to twenty weeks pregnancy.

(2) Later antenatal cases, from twenty to twenty eight w eeks pregnancy.

(3) Late antenatal cases, from twenty nine weeks to term.

(4) Postnatal cases, taken after the eighth day of the puerperium.

The results of the glycogen test were as follows:-

(1) In the first group, fifty seven per cent. of smears examined were positive.

(2) In the second group, sixteen per cent. of smears were positive.

(3) In the third group, twenty four per cent. of smears were positive.

(4) In the fourth group, fifty per cent. of smears were positive.

As there seems no correlation between the groups, judged on the above results, it would seem that no satisfactory conclusions can be drawn from this method of investigation.

In the second method the smears, having been taken, were immediately fixed in a mixture of equal parts of 95 per cent. alcohol and ether for fifteen minutes. They were then rinsed successively in 70 per cent. and 50 per cent. alcohol and distilled water.

The slides were then stained in Harris Haematoxylin for 1½-2 minutes. They were then placed for one minute in a solution of 97 ml. of 70 per cent. alcohol, to which had been added 3 ml. of strong solution of ammonia. The slides were then passed through two changes of 70 per cent. alcohol, and then passed successively through 80 per cent. and 95 per cent. alcohol.

The slides were then stained for three quarters of a minute in OG 6, and were then rinsed eight times in each of two jars containing 95 per cent. alcohol, and one jar of absolute alcohol.

Finally the slides were rinsed in xylol, and mounted in DPX.

With this stain, the nuclei stain a dark

purple colour. The basophilic cells stain a green to a blue-green colour. The acidophilic cells stain from a pink to an orange colour. The presence of these acidophilic cells, indicates a positive oestrogen reaction.

The smears examined were divided into the same four groups as were the slides exposed to iodine vapour. From each group, six slides were examined, a total of one hundred cells being count ed on each slide. These hundred cells were divided into basophilic and acidophilic types as previously described.

<u>These results were as follows</u>:-

In the first group - Acidophilic cells: 33.8 per c ent.
Basophilic cells: 66.2 per œnt.

In the second group - Acidophilic cells: 35.5 per cent.
Basophilic cells: 64.5 per cent.

In the third group - Acidophilic cells: 76 per c ent.
Basophilic cells: 24 per c ent.

In the fourth group - Acidophilic cells: 44 per œnt.
Basophilic cells: 56 per c ent.

As the presence of acidophilic cells indicates a measure of oestrogenic activity, the above results indicate that oestrogenic activity is relatively low in early pregnancy, rises to a significant extent in late pregnancy, and again falls in the puerperium.

Furthermore, these results are in accord with the findings of the investigation into the activity of the ovary during the different stages of pregnancy.

A summary of opinions held regarding ovarian activity in pregnancy, and the conclusions reachedfrom the work embodied in this thesis.

The commonly held opinion is that, apart from the corpus luteum, the ovaries are inactive during pregnancy, after the twelfthweek. The same opinion has been held in the case of animals, and Beard (1897) and Prenant (1898) believed that ovulation was suppressed, as part of the mechanism for the protection of the embryo. From time to time, evidence has appeared as to the presence of ovarian activity, occurring in pregnancy, in animals. The existence of waves of follicular growth in the pregnant guinea pig was noted by Loeb (1910), while the onset of oestrus was noted as occurring five times in one pregnant rat (Nelson, 1929). Swezy and Evans (1930) noted that the rhythmical production of new follicles, which occurs during the normal oestrus cycle of rats, was not interrupted by pregnancy, but continued with the same four or fiveday rhythm.

In the oestrus cycle of rats, Swezy (1933) has described three main reactions. These

consist of (1) the ripening of Graafian follicles, (2) the conversion of the follicles into small corpora lutea or lutein cysts, and (3) the formation of lutein cells from the germinal epithelium. Swezystated, that in association with the oestrus cycles during pregnancy, there is the ripening of a large number of follicles which may exceed the number appearing in the ovary in oestrus uncomplicated by pregnancy. The smaller follicles may become atretic before they have grown very big, while many of the larger ones are said to be converted into corpora lutea not larger than one third of the size of pregnancy corpora. The corpora lutea cysts are formed only sometimes, especially during mid or late pregnancy. The granulosa cells degenerate and large lutein cells are formed mainly from the theca interna.

The small corpora in the rat, are thought to be formed from the medium sized follicles.

Swezy maintains that the formation of "luteincells", directly from the germinal epithelium or tunica albuginea, without an intermediate growth phase, is regarded as

another characteristic of the pregnant ovary, as they are rarely seen in the ovaries of non-pregnant rats.

Zuckerman and Parkes (1932) drew attention to the atresia which overtakes many resting follicles in the ovary of the pregnant baboon.

In the human ovary, G illman and Stein (1941) recorded the presence of numerous atretic follicles, and it was suggested that there was unmistakable evidence of an ovarian rhythm during pregnancy. The ovary was certainly not in an inactive state.

If activity exists in the human ovary during pregnancy, this must be in response to some hormonal stimulation. Many investigations have been carried out, as to the type and origin of these hormones, with special reference to the gonadotrophins.

A great deal of work has been carried out on the quantities of gonadotrophins found in the placental blood and the urine of pregnant women, (Aschheim, 1926, 1927; Aschheim and Zondek, 1927; Zondek and Aschheim, 1927; Fels, 1927; Frank,

1935; Evans, 1936; Evans, Korpi and Simpson, 1936; Smith, 1937; Evans, Kohls, and Wonders, 1937; Zondek, 1938; Smith and Smith, 1939, 1940, 1941; Wislocki and Bennett, 1953; Stroink and Muhlbock, 1948; Agadzhanoff, 1953).

Many authorities think that the gonadotrophins are pituitary in origin (Zondek, 1932; Evans, Myer and Simpson, 1932; Schockaert and Siebke, 1933; Kunkel ald Loeb, 1935; Fremery and Scheygrond, 1937; Witschi, 1937; Govan and Mukherjee, 1950; Lajos, Szontagh and Pali, 1953; Calapa, 1954), although others believe them to be of chorionic origin (Smith and Smith, 1939; Loraine and Matthew, 1950).

There is a very marked increase in the production of gonadotrophins during pregnancy, and especially during pregnancy complicated by toxaemia. Smith and Smith (1939, 1940, 1941) believe that this excess of gonadotrophins is placental in origin. In respect of this idea, Stewart, Sano and Montgomery (1948) state that they have produced gonadotrophins from tissue cultures of three month old human placenta. The amount of

of gonadotrophin secreted, is said to be directly related to the growth of Langhans cells. However, it is well-known that in toxaemia the placenta is degenerate, and often does not show the presence of any Langhan's cells. This would be a point in favour of an extra-placental origin of the gonadotrophins, possibly the pituitary (Govan and Mukherjee, 1950).

Although experiments have shown that gonadotrophins produce a reaction in the ovaries of animals, the commonly held opinion is that they have little or no effect upon the human ovary. (Giest, 1933; Pratt, 1933; Hamblen, 1939; Brown, Bradbury and Metzger, 1941).

Hinglais and Hinglais (1949) report the results of hormonal studies on a case of chorionepithelioma with metastases. The levels of prolan-A and prolan-B excretion rose progressively over a period of eighteen months. The excretion of prolan-B rose from 300 units per litre to 100,000 unitsper litre. The urinary oestrogens and pregnanediol, remained at a low and almost unvarying level. The authors inferred from this that chorionic

gonadotrophins, whichstimulate the ovaries of various animals, are, by contrast, relatively or entirely inactive in the secretion of oestrogens and progestin in the human ovary, and that chorionic tissue does not appear to produce the oestrogenic and progestin principles.

Although it seems probable that chorionic gonadotrophins do not act, to any marked extent, upon the human ovary, nevertheless, the work embodied in this thesis, would indicate that there probably is ovarian activity during pregnancy.

As stated previously, a commonly held opinion is that the corpus luteum ceases to function around the twelfth week of pregnancy, and thereafter undergoes degeneration and disappears. If this were the case, and if all ovarian function ceased, then, histological evidence of ovarian activity should be lacking.

In the early pregnancy series, a corpus luteum was present in four cases out of seven, which is fifty seven percent. In two of the sections showing corpora lutea, the corpus luteum was markedly degenerate, while in the

other two, only slight degenerative changes were seen. In one of these, the pregnancy was of eight weeks duration, while the other was of sixteen weeks duration.

In both these early pregnancy series, both fully developed and atretic follicles were seen. Even more important is the fact th at the atretic follicles were present in number, and their lining theca interna indicated the probability of ovarian activity, by the degree of proliferation, and the presence of increased vascular change.

At this early stage in pregnancy, interest is aroused, as to the hormonal control of any probable ovarian activity. Oestrin excretion in the urine, is low during the menstrual cycle. It undergoes a gradual increase during pregnancy. Maximum values are reached shortly before parturition, unlike the excretion of chorionic gonadotrophins.

It may be that oestrin is excreted by the ovary, at the beginning of pregnancy, and that the excretion is then carried on by the placenta.

As has been discussed previously, the decline in placental efficiency, and its gross changes as it ages, indicates that the excretion of gonadotrophins, at least, is carried on by the pituitary.

As stated in the introduction, comparatively little work had been done on the excretion of progesterone. Its metabolic product, pregnanediol, on the other hand, has been estimated by many workers (Browne, Henry and Venning, 1937; Jones and Weil, 1938; Cope, 1940; Bachman, Leekley and Hirschmann, 1940; Tien, 1941; Talbot et al., 1941; Astwood and Seegar Jones, 1941; Guterman, 1944, 1945, 1946; Venning, 1946; Jayle and Libert, 1946; Dahle, 1946; McCormack, 1946; Somerville, Gough and Marrian, 1947; Bender, 1947; Bishop, 1949; Bull.Soc.Ital. Biol., 1952; Bradshaw, 1953).

Progesterone appears to be secreted by the ovary in early pregnancy, but later in pregnancy, it appears to be secreted by the placenta (Robson, 1936; Seegar and Delfs, 1940; Wislocki, Dempsey and Fawcett, 1948).

Like progesterone, oestrogens have been found in the placenta, in early pregnancy (Stroink and Muhlbock, 1948; Wislocki, Dempsey and Fawcett, 1948; Stewart, Sano and Montgomery, 1948).

As suggested, many of the steroids formerly thought to be secreted by the placenta, may instead be secreted by the ovaries, if ovarian function continues throughout pregnancy.

In this thesis an attempt has been made to show that ovarian function does continue throughout pregnancy, and until parturition. The presence of ovarian activity was shown by the following features:-

(1) The presence and state of the corpus luteum.

(2) The presence of developing follicles, from the early primordial type, to the fully developed follicle.

(3) The number and type of follicles undergoing atresia.

(4) The condition of the theca interna, as seen both in the walls of the follicles undergoing atresia, and also in the areas of thecal change.

These various features were described in

four groups of cases. There were:-

(1) Seven patients dying in early pregnancy, the latest being at eighteen weeks.

(2) Eighteen patients subjected to laparotomy for ectopic gestation. All of these occurred before eighteen weeks pregnancy.

(3) Forty two patients dying in late pregnancy. While the majority of these cases were at term, others occurred at different durations from thirty to forty weeks, and one patient was as early as twenty eight weeks.

(4) Twenty patients subjected to laparotomy for Caesarean section. While the majority of these patients were at term, two were as early as thirty five weeks pregnancy.

In the early pregnancy series, corpora lutea were found to be present in over fifty per cent. of the cases, showing slight degenerative changes in half the sections examined. Corpora albicantia were usually present in varying numbers and sizes, either in addition to, or without, the presence of a corpus luteum. In a few instances corpora lutea were

present without any corpora albicantia.

Developing follicles were present in six out of the seven specimens examined. The number present varied from nil to thirty one, and these were all early primordial in type.

One section showed the presence of one fully-developed follicle, while another showed one relatively far advanced follicle.

Atretic follicles were present in all except one of the sections examined. The number present varied from nil to eight and all of them were lined with theca interna. Granulosa was present, in addition, in a number of the cases.

The theca interna showed proliferative changes in five out of the seven specimens examined, with, in addition, increased vascularity, in two of the sections.

There were no areas of thecal change in the seven early pregnancy cases.

The eighteen specimens obtained at laparotomy for ectopic gestation, showed, in essence, the same featu res as in the seven early pregnancy cases. The theca interna showed

proliferative changes in five out of the eighteen specimens examined. Increased vascularity was present in one case, without an accompanying proliferative change.

In the forty two late pregnancy cases a corpus luteum was present in twenty eight of the sections examined. It did not show any degenerative change in two cases; showed slight degenerative change in seventeen cases, and marked degenerative change in nine cases.

Primordial follicles were present in all stages of development. The number of early primordial types varied from nil to two hundred and ninety five, the average number per section being thirty four.

The average number of further advanced follicles was 2 per section.

The average number of fully developed follicles was 0.9 per section.

The average number of pat ent atretic follicles was 4.1 per section.

The average number of collapsed atretic follicles was 2.7 per section.

The average number of thecal areas was 1.6 per section.

The follicles undergoing atresia were all lined with theca interna, granulosa also being present in some of the sections.

In addition to that lining the follicles, there were also areas of thecal change, which were not clearly identifiable as follicles. The condition of these thecal areas varied as did that of the theca interna lining the follicles.

An analysis of the theca interna, showed the following results:-

Proliferative change was present in twelve of the sections examined.

Increased vascularity was present in three of the sections examined.

Both proliferative change and increased vascularity were present in sixteen of the sections examined.

The theca interna showed degenerative change in 16.6 per cent. of the sections examined. The features of the six specimens showing the greatest

activity, were described.

With relation to the clinical conditions of these patients, it was found that out of the whole group, the number of cases not showing toxaemia, was just twice the number who had toxaemia.

Of the six showing the greatest activity, the number of toxaemia cases was exactly the same as the number of cases not showing toxaemia.

In the twenty cases obtained at laparotomy for Caesarean Section a corpus luteum was present in five of the sections examined. It did not show any degenerative change in four cases, and slight degenerative change in one case.

Primordial follicles were present in all stages of development. The number of early primordial follicles var ed from nil to one hundred and ninety six, the average number per section being 20.5.

The average number of further advanced follicles was 0.65 per section.

The average number of fully developed follicles was 0.1 per section.

The average number of patent atretic follicles was 1.7 per section.

The average number of collapsed atretic follicles was 1.2 per section.

The average number of thecal areas was 0.75 per section.

A description of the follicles undergoing atresia was the same as in the forty two late pregnancy cases.

An analysis of the theca interna, showed the following results:-

Proliferative change was present in sixteen of the sections examined. Increased vascularity was present in four of the sections examined. Both proliferative change and increased vascularity were present in four of the sections examined.

The theca interna showed degenerative change in 5 per cent. of the sections examined. The features of the four specimens showing the greatest activity were described. With relation to the clinical conditions of the patients in this group, it was found that out of the whole group, the number of cases not showing toxaemia,

was about six times the number who had toxaemia.

Of the four showing the greatest activity, the number of toxaemia cases was exactly the same as the number of cases not showing toxaemia.

In the experimental work it was decided to test the hypothesis that since chorionic gonadotrophin does not appear to have much influence on the human ovary, the findings obtained in the thesis, would suggest that some other gonadotrophin is present during pregnancy, and its activity increases, as term approaches.

On the assumption that these gonadotrophins are excreted in the urine, extracts were prepared from the urine of patients at various stages of pregnancy, avoiding any patients who might be suffering from toxaemia of pregnancy. In both the early and the late pregnancies, extracts were made, using both kaolin and benzoic acid.

A synopsis of the methods used, in the preparation of these extracts, was given.

The rats injected with early pregnancy urine, were divided into two groups, one being injected with the kaolin extract of the hormone, and the

other group with the banzoic acid extract.

The sections prepared were studied with regard to the following:-

(1) Primordial follicles.

(2) Atretic follicles.

(3) Corpora lutea.

(4) Stromal activity.

Primordial follicles and atretic follicles were found to be present in varying numbers.

Corpora lutea were found to appear, as early as forty eight hours after injection, and were present in most of the groups, up to one hundred and twenty hours after injection.

Stromal activity was found to be present throughout both the groups.

The rats injected with late pregnancy urine, were divided into three groups, one being injected with the benzoic acid extract, and the other two with kaolin extracts.

The sections prepared were studied in the same way as in the early pregnancy group. The description of the primordial and the atretic follicles, was found to be the same as in the

early pregnancy series.

There was a marked difference, both in the number of corpora lutea, and also as regards the time when they appeared.

In the benzoic acid series, only one small corpus luteum appeared in the group which were killed after seventy two hours.

In the kaolin series, only one corpus luteum appeared in each series. Both of them appeared in specimens killed after one hundred and twenty hours.

Stromal activity was found to be present throughout all three groups.

By contrast, stromal activity was found to be absent in the control series of rats.

The conclusions drawn from these experiments, were that in the case of early pregnancy urine, there was a hormone that played a part in the development of corpora lutea. This hormone seemed to be missing from the urine taken from late pregnancy cases. It seemed possible that in late pregnancy the hormone itself did not cause the formation of corpora lutea, but rather that

it set in motion a train of events, which stimulated the animals own pituitary to produce luteinising hormone.

The clinical investigation gave inconclusive results, with regard to the smears treated to show the presence of glycogen. The smears stained by the method of Papanicolaou, tended to confirm the fact that oestrogenic activity rises throughout pregnancy, and falls after parturition.

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